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CYTOLOGICAL STUDIES ON THE NUCLEAR DIVISION OF THE POLLEN MOTHER-CELLS OF SOME CEREALS AND THEIR HYBRIDS.

BY

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(With Plates X—XIII)

At no time in the annals of botany has the problem of heredity received such a high degree of attention from the scientific world as in recent years, investigations being carried on, on the one hand through experiments on hybridation, and on the other through cytological studies on the division of sexual nuclei.

The existence of reducion division in plants has been a subject for a long time. Even at so recent a time as 1900, the question that there is no such thing as reduction division in the vegetable kingdom, was considered to have been settled definitely (Strasburger 1900). However the existence of reduction division in animals, and the rediscovery of Mendel's law on the hybridation made a renewed study of the subject necessary. Farmer and Moore ('03) advanced the "loop theory" in which they considered the first division as the reduction division, this opinion being supported by Strasburger ('04), Gregory ('04), Williams ('04), etc. On the other side Grégoire ('04), Berghs ('04) and Allen ('05) advocated the "separation theory", all these investigators agreeing in their recognition of the existence of the reduction division in the first division of the gametogenesis, supported by Miyake ('05), Overton ('06), Strasburger ('05), etc. Recently Mottier ('07, '09), Gates ('08, '09), Geerts ('03), Lewis ('08), Davis ('09), and Schaffner ('09) endorsed the former theory, while Grégoire ('07), Overton ('09), Strasburger ('08 '09) Rosenberg ('09, '09) and others supported the latter theory.

Besides, cytologists do not agree with one another on the phases preceeding the synapsis which are important in the investigation of the subject. Some are of the opinion, that the "prochromosomes" are always present in the resting nuclei, but others that the chromatin gathers about gamocentrums before synapsis, while still others entertain the opinion that there are no such bodies and that the reticulum changes directly to the chromatin threads before synapsis.

The results ef my studies have led me to stand by the "separation theory". In the nucleus no prochromosomes are at first visible in a perfect resting state, but they

gradually appear afterwards, accompanied later by other chromatic granules, both of them increasing in size, completing the reticulum, and finally entering the synaptic stage.

The materials which I have studied are *Triticum vulgare*, *Hordeum distichon*, *Secale cereale* and the hybrids between *T. vulgare* and *S. cereale*.

They were fixed with Flemming's mixture of chromic, osmic and acetic acid, or sometimes also with acetic alcohol. The sections were cut to 5μ in thickness or when necessary 2–3μ as at the pre- and post-synapsis. They were stained with Heidenhain's iron-alum haematoxylin combination.

Somatic Nucleus.

Before I enter into the discussion of the spermatogenesis, let me briefly describe the somatic nucleus of these cereals.

Rosenberg ('04, p. 254 ff.) observed chromatic grains in the resting vegetative nuclei of Capsella, Zostera and Calendula, and called them "Chromatin Körner". Their number is equal to that of the chromosomes. He considered that the chromosomes do not entirely disappear in the perfect resting nucleus, but take the form of grains, thus making them the essential permanent part of the nucleus.

Overton ('05, p. 123 ff.) observing also similar chromatic grains in Thalictrum, Helleborus, Podophyllum, Calycanthus, Campanula, and Richardia ('09, p. 34 ff.), named them "prochromosomes" with the same opinion. Recently while studying the resting nuclei of Crepis ('09, p. 65 ff.) and Drosera ('09, p. 17 ff.), Rosenberg observed their prochromosomes pairing by twos, and in the nucleus of the Drosera hybrid 10 pairs and 10 single ones.

I studied the nucleus of the wall-cells of ovaries and of anthers of the cereals under investigation, and found that the conclusions reached by the researches of Rosenberg and Overton correspond exactly with those of my studies, except for the fact, that in the stage previous to the so-called resting state, the nucleus has no particular structure, being stained somewhat deeper than the cytoplasm around it, and having only one large nucleolus (Pl. X. Fig. 1). There appear gradually and not simultaneously small chromatic bodies, whose number increases until it reaches about that of chromosomes. They are the so-called prochromosomes. They increase gradually in size and assume several shapes (Pl. X. Fig. 2). Afterwards there appear in great abundance small chromatin granules which are stained somewhat lighter in a group or scattered groups in the nucleus. These granules are distributed throughout the whole cavity of the nucleus and form the reticulum (Pl. X. Fig. 5). By observing this reticulum closely, the difference between linin and chromatin may clearly be made out contrary to Grégoire's opinion that chromatin is only a

knot of linin. In short, the chromatin granules seem to be associated with linin, while the prochromosomes, which are nothing but a specialized form of chromatin, appear independently of linin. With the growth of the nucleus, chromatin increasing in size and number and connected with linin, forms the chromosomes in prophase. At this time, the prochromosomes are the largest of all chromatin bodies, and seem to become the centres of new chromosomes, to one side of which the adjoining chromatin granules are fused in a serial line. Thus as Rosenberg has already stated in the case of Drosera hybrid, the prochromosomes in our case show also the nature of polarity (Pl. X. Fig. 5). In some cells, especially in the archesporium cells, new chromosomes, being very fine and long, present an appearance somewhat similar to that of the threads of synapsis in the pollen mother-cell. However, there are not to be seen parallel threads characteristic of the latter (Pl. X. Fig. 3).

The above mentioned facts were demonstrated in all my materials, such as barley, wheat, rye and wheat × rye.

Reduction Division in Hordeum distichon, Triticum vulgare and Secale cereale.

Pre-synaptic Phase.

The pre-synaptic phases of the pollen mother-cells resemble those of the somatic cells. In the pollen mother-cells these phases are very distinct, and their stages from the youngest to the oldest may easily be observed successively in the same sections.

At first the nucleus of the pollen mother-cell has only one large nucleolus and neither prochromosomes nor chromatin granules are observable. This does not agree with Thalictrum, Calycanthus and Richardia studied by Overton, who recognized in these plants the permanence of chromosomes. Prochromosomes appear granually one by one until they reach a definite number, i. e., about twice the reduced number of the chromosomes (Pl. X. Fig. 7, 8, 32; Pl. XI. Fig. 55). They are at first minute spherical granules, but they increase in size and assume somewhat irregular shaped bodies. The young spherical prochromosomes usually lie near the periphery of the nuclear cavity, and they are seen often in pairs (Pl. X. Fig. 32, Pl. XI. Fig. 55). At this time no other chromatin nor linin exists. With the growth of the nucleus, other kinds of chromatin, which are smaller and lighter stained appear in the form of granules having different shapes and sizes. These chromatic granules form the reticulum, being connected with one another by the linin which is attached at one or several points of the granules (Pl. X. Fig.10, 34). The chromatin granules increase gradually in size and number. The formation of the reticulum is per-

fected, as the prochromosomes are becoming obscure. The chromatin bodies in the reticulum are often arranged in pairs (Pl. X. Fig. 11, 12, 35).

The nucleolus increases also in number, two or three being usual number. The nucleolus, to which large and deeply stained chromatin bodies are attached, is generally lightly stained. One may deduce from this phenomenon that chromatin bodies are fed by nucleolus, and that the increase of nucleoli in number is for convenient distribution of nutriment.

When the reticulum is completed it contracts into synapsis. As the reticulum is contracting, conspicuous changes take place in chromatin bodies. They are finely divided and more or less evenly distributed in the linin ground, forming nuclear threads. The contraction takes place towards the spot where the chromatic substance is most abundant (Pl. X. Fig. 11, 12, 35, 36). In wheat the contraction takes place after the reticulum is almost completely changed to threads (Pl. XI. Fig. 58, 59), two of which generally run parallel. In these parallel threads two pairing chromatin bodies are to be seen (Pl. XI. Fig. 59). Thus in pre-synapsis, what I have observed in the formation of chromatin granules is different from the cases described by Rosenberg in Crepis ('09, p. 67 ff.), and in Drosera ('09, p. 22 ff.), or by Overton in Thalictrum, Helleborus, Calycanthus, Campanula ('05, p. 137 ff.), and Richardia ('09, p. 34 ff.). Nor have I observed in my materials anything like Miyake's gamosomes found in Galtonia, Iris, Lilium, Allium, Funkia and Tradescantia. But my results agree rather with those of Allen ('05, p. 190 ff.) of Mottier ('09), who studied in Lilium, of Grégoire ('07, p. 388 ff.) in Lilium and Allium, and of Davis ('09) who lately studied in Oenothera and found the presence of prochromosomes and the formation of reticulum, which are essentially the same as I have described above. However, the prochromosomes did not exist in my materials at first, but appeared afterwards.

Synapsis.[1]

The contraction of nuclear content to a ball is by Moore ('95) named synapsis, meaning the conjugation of parental chromosomomes. The phenomenon had also been observed previously by several authors. Some authors considered it as artificially produced, while others thought it resulted by gravity.[2]

Strasburger ('04, p. 604 ff.) holds that synapsis is the most important period in the development of the heterotypic division, and in the year 1905 he, together with his pupils Allen, Miyake, and Overton tried to demonstrate it. Miyake ('95, p. 89 ff.) declares that

(1) Treatises on synapsis are well referred in Miyake's ('05) and Overton's ('05) papers.

(2) Cardiff ('06).

gamosomes, after having made reciprocal action in synapsis, stretch to gamomites; Overton ('05, p. 137 ff.) states that gamosomes lengthen into gamomites before or after synapsis in which also reciprocal action takes place; while Allen found that the reticulum changing into chromatin threads enters into synapsis, and in post-synapsis paired chromatin threads conjugate and homologous chromomeres in the threads fuse also. Allen considered this process to be the conjugation of parental chromosomes, giving reciprocal influence,—a view supported by Strasburger ('05, p. 40 ff.), Overton ('09, p. 46 ff.) and others.

There were often chromatic masses, in my materials, in the early stage of synapsis, but they were not regular in number or size as gamosomes, and could not be considered as similar bodies as gamosomes. Soon afterwards all nuclear elements change to uniform threads in the synapsis. The threads, two of which run parallel, have chromatic granules imbedded at more or less regular distances, presenting somewhat the appearance of pearl-strings. This nature can not be easily recognized in the synaptic ball, but in a tangential-section of the ball or in the loosening chromatin threads it is very easily seen (Pl. X. Fig. 13, 14). In barley it is typical, no chromatic masses remaining in the synaptic ball and the structure of chromatin threads being very distinct. In wheat the threads are typical as in barley, but they conjugate at the stage just previous to synapsis. In rye the synapsis is not typical. These are often many chromatic masses remaining in synaptic balls. Sometimes some chromatic masses are found in such a condition as if they were migrating to neighbouring cells (Pl. X. Fig. 37). This phenomenon was first observed by Koernicke ('01) in the pollen mother-cells of some plants and lately by Digby ('09) in the pollen mother-cells of Galtonia. But at the present time, cytologists are inclined to believe that it is caused by bad fixing, and Rosenberg ('09, p. 22) also holds it as an artificial product in his studies of Drosera. In rye, the phenomenon is visible only in certain preparations, while in others it can not be seen. From this fact I consider, that it must be an artificial product, being caused through bad fixation or mechanical injury at the time of fixing.

The nuclear threads in the post-synapsis are always uniform. Some authors describe them as single threads while others as double ones. In wheat, the paired chromatin threads have already conjugated just before or in synapsis, retaining the single nature at the post-synapsis (Pl. XI. Fig. 60), while in barley and rye, conjugation having not yet taken place, the double nature is still retained (Pl. X. Fig. 14, 15, 16).

If we consider the significance of synapsis from these facts, we may conclude that the contraction is for the sake of the three following points; namely, of the reticulum to transform into threads, of the equilibrium to be gained by hereditary substances, and of the reciprocal influence of homologous parental, paternal or maternal, hereditary substances. In barley and rye in which two parallel nuclear threads do not conjugate during synapsis,

its meaning may be chiefly either of the last two cases.

Spirem.

Nuclear threads in a synaptic ball become loosened gradually and are distributed in the nuclear cavity. This is the stage of "hollow spirem" (Pl. X. Fig. 17, 39; Pl. XI. Fig. 61, 62). At the time of passing to spirem from synapsis, as Allen describes, paired chromatic threads conjugate and homologous chromomeres fuse up in the case of barley and rye. In wheat this process takes place previously, immediately before or in the synapsis, and at this stage only one spirem can be seen. Grégoire ('04; '07 p. 399 ff.) argues that there is no actual fusion or conjugation of filaments, but in my materials, paired filaments fused up entirely. As the spirem is growing, the conjugated filament is again separated into paired threads (Pl. X. Fig. 18, 40; Pl. XI. Fig. 42).

At a later stage of the spirem, the filaments become larger and shorter. This has been described as the second synapsis or second contraction by some authors, and in this stage no fusion between paired filaments takes place, contrary to the opinion of Farmer and Moore ('03, '05) and Mottier ('07, '09) (Pl. X. Fig. 18, 19; Pl. XI. Fig. 64). I am of the same opinion with many authors in holding that the so-called "second contraction" is not characteristic of maturation divisions in general and that it has no special significance.

There are two explanations for the process of the union and separation of the two nuclear threads stated above. The one was proposed by Farmer and Moore ('03, '05,) and Mottier ('07, '09) who hold that two nuclear threads are formed by a longitudinal fission, and the other by Grégoire ('04, '07), Berghs ('04), Allen ('05), Strasburger ('05, '08, '09), Miyake ('05) and Overton ('05, '09), who are of the opinion that two chromatic threads originate from mother and father chromosomes, which conjugate in spirem and afterwards again split into two filaments. I support the latter view, as it will be fully stated later on.

Formation of double Chromosomes.

About the formation of double chromosomes divergent opinions are held by authors. A theory prevailing about the year 1900 is that a spirem, which has a longitudinal fission, is segmented transversely and produces double chromosomes in reduced number. The supporters of this theory were Sargant, Guignard, Grégoire, Strasburger, Mottier, etc. Another theory proposed by Bejaleff, Ishikawa, Atkinson, etc., is that the reduction occurs in the second division, but they agree with the first theory in the formation of the double

chromosomes. On the other hand, Schaffner, Dixon, etc. proposed that the double chromosomes arise through looping.(1) Farmer and Moore ('03) published a treatise supporting the loop-theory, and threw a new light on the question of the reduction division, followed by Gregory ('04), Williams ('04), Strasburger ('04), Mottier ('07, '09), etc. However Grégoire ('04) Berghs ('04), and Allen ('05) studying this problem anew came to the conclusion, that two filaments are not produced by fission, but by separation of two nuclear threads, which have conjugated in the post-synapsis; and it was supported by Rosenberg ('05, '09), Strasburger ('05) Miyake ('05) Overton ('05), etc. Recently, Yamanouchi ('09) in Fucus, Hyde ('09) in Hyacinthus and Mottier ('07, '09) in Lilium confirmed the loop-theory; while Gates ('08), Geerts ('09) and Davis ('09) in Oenothera, and Schaffner ('09) in Agave are on the side of Farmer and Moore's theory. Some authors, for instance, Montgomery ('98), Yamanouchi ('09, '10), and Gates ('09) believe that two modes of formation of the double chromosomes—the looping and separation—exist in the plant kingdom.

I studied this point with great care, tracing the process thoroughly; and I am fully convinced, that the separation-theory is correct, at least, in the case of cereals used in my study. In barley I was able to confirm this point most easily and plainly, as is to be seen in Fig. 20 and 21 of Pl. X. The process in wheat and rye, although not so plain as in barley, is still easily traced (Pl. XI, Fig. 43, 44, 66, 64).

Diakinesis.

In barley the twisting double chromosomes loose themselves and becoming thicker and shorter, present elliptic or circular rings (Pl. X, Fig. 22). In rye the diakinetic figure is very curious. Each double chromosome often connects with others forming irregular groups or rings. Such cases were already observed in Galtonia by Miyake ('05, p. 99) in which, however, the single chromosomes connect with one another at their ends. And in rye many small nucleoli, besides one large one are present, and attached mostly to chromosomes. They keep the staining power sometimes long after the time, when the large one has lost it, and even to the period of mitosis. The small nucleoli seem to be formed from about the stage of early spirem (Pl. XI. Fig. 41). In wheat I could not obtain a good preparation for diakinesis. According to Koernicke's descriptions and my own observations, however, I can state that the diakinetic figures are similar to those of barley, except the richness in chromatin (Pl. XII. Fig. 64).

In wheat and barley, one or two large nucleoli are present, which have become lightly stained at the end of diakinesis; the many small ones do not exist as in the case

(1) Literatures relating to this subject are found in Miyake's paper ('05, p. 94 ff.).

of rye.

The role which nucleoli play in the nuclear division, is not yet clearly determined. Strasburger ('05 p. 33 ff.) holds that it is consumed for the spindle-formation. Some authors agree with him, while others believe that it is consumed for the chromosome formation. Some authors, as Miyake, consider that the greater part is used for the spindle formation and the remaining part for the chromosomes. I observed the chromatin attached to a lightly stained nucleolus to be distinguished from others both in size and staining power. Such a chromatin is always larger and is deeper stained than others (Pl. X. Fig. 11, 35). Smaller nucleoli which are attached to nuclear filaments at spirem or diakinesis in rye seem to nourish them. From these facts I may conclude that it is for the most part used for the growth of chromosomes, and the remaining discoloured ground body may be changed to the spindle, against Strasburger's opinion, who believes that there must be a great change of matters in the formation of the lighter stainable spindle from the deeper stainable nucleolus.

The First and Second Divisions.

In wheat, as described by Overton ('93) and Koernicke ('96) and in rye, the reduced number of chromosomes is 8, while in barley 7. The reduced number of chromosomes is generally even. The odd number as in the case of barley is at present only in a few examples as in *Oenothera* (7 except *O. gigas*), in *Crepis virens* (3), *Stenophragma* (5), and in *Allium Moly* (7).

In barley, the spindle fibres are attached to the chromosomes at the middle portion (Pl. X. Fig. 23, 24). Therefore the bivalent chromosomes drawn apart assume a rhombic shape and separate similarly at both ends. But sometimes they separate first at one end, thus giving rise to the figure as represented in Fig. 23 of Pl. X. The longitudinal fission for the next division is visible often in anaphase (Pl. X. Fig. 26), and almost always in the pole of the spindle, where bent chromosomes come in contact at their backs (Pl. X. Fig. 27). The chromosomes in the pole become alveolated gradually, as Grégoire first discovered. Many minute angular chromatic grains or extra-nucleoli are recognised in the cytoplasm of the pollen mother-cells and also vegetative cells of barley (Pl. X. Fig. 25, 26). Their behaviour during mitosis, though I have observed them with great care, shows no definite phenomenon as in Crepis, which was studied by Tawara ('10). In wheat, the chromosomes, which are drawn to the poles without changing their shape much, are a more or less irregular chromatic mass, in which the alveoration takes place gradually (Pl. XI. Fig. 66, 67). In rye the double chromosomes come to the equatorial plate joined with one another as already stated, of which the numbering and the mode of separation

are difficult to make out (Pl. XI. Fig. 48), on account of the abundance of chromatin. The chromosomes in the poles of the spindle do not alveolate as in barley and wheat, but turn to spirem and are arranged as in Fig. 51 of Pl. XI.

Now, the question is whether chromosomes in the daughter nucleus alveolate or stretch to spirem. Strasburger, Mottier, etc. held the latter opinion, while Grégoire and Wygærts advocated the former view, which seems to prevail more at present.(1) According to my own observations, however, in barley and wheat, they alveolate, while in rye they turn to spirem, indicating the existence of these two modes even in plants belonging to the same family.

In all my materials the homöotypic division occurs as usual, in which also the number of chromosomes is easily counted, especially in wheat and rye, whose rich chromatin hindered the observation in the first division (Pl. XI. Fig. 52, 53; Pl. XII. Fig. 68, 69). The chromosomes, which form the grand-daughter nucleus alveolate or turn to spirem in a similar manner as in the case of daughter nuclei (Pl. X. Fig. 31; Pl. XI. Fig. 54; Pl. XII. Fig. 70).

Reduction Division in Wheat × Rye.

The heterotypic division in plant hybrids began to receive great attention from cytologists, since the publication of Rosenberg's paper on Drosera-hybrid in 1903, and many important facts have been discovered. Tischler ('06, '06, '08) came to the conclusion that the absence of fertilizing power in the pollen of several hybrids is not caused by the abnormal reduction division, but by their degeneration based on the deficiency of cytoplasma. Rosenberg ('03, '04, '09) studied the Drosera hybrid—*Drosera longifolia* (40 chromosomes) × *D. rotundifolia* (20 chromosomes)—and found 20 double chromosomes and 10 single chromosomes at reduction division. Gates ('09) avers that in the hybrid *Oenothera lata* (14 chromosomes) × *O. gigas* (28 chomosomes) there arises 20 double chromosomes and a single one, or simply 20 double chromosomes.

The wheat-rye hybrid, which I have studied, was crossed by Mr. Masanao Ito in the summer of 1908 in the College Experimental Field. The seeds thus obtained were sown in 1909. The outer morphology of this hybrid resembles closely that of Tschermak ('07, p. 144 ff.). He ('04) discovered that the hybrid does not produce any pollen and is sterile, but our hybrid has a few pollens, which have folds on exine. Most of the pollen had already been degenerated. From the results of our pollinating experiments, we have to agree with Tschermak in considering our hybrid to be also sterile, when pollinated with

(1) These literatures are found in Miyake's paper ('05).

its own pollens. To test the ferility of the egg-cell, we pollinated the hybrid with the pollen of rye and wheat, but the result was negative. Notwithstanding, we found a few seeds, generally one seed to a few ears. We can not say whether it is the second generation of the hybrid, or the hybrid fertilised by one of the parents' pollen.(1)

Cytological features of the hybrid resemble in essential points those of barley and the parent plants described above.

In the formation of reticulum some prochromosomes unite and form several large irregular chromatic masses, which lie in one portion of the nuclear cavity; while the reticulum appears in another portion (Pl. XII. Fig. 71, 72). Later on, prochromosomes and reticulum contract into synapsis, containing the prominent chromatic masses in its interior (Pl. XII. Fig. 74). But there can often be seen the common reticulum and synapsis also (Pl. XII. Fig. 75). Although in Mirabilis-hybrid studied by Tischler ('08 p. 39) the synaptic duration is very short, in our hybrid the synapsis lasts very long, even to the metaphase. The synaptic mass shows the spirem structure in the thinly cut sections (Pl. XII. Fig. 77). The abnormal synapsis may be caused by one or both of the following facts, viz. the poor nourishment for pollen mother-cells, and the difficulty in the process of the conjugation of the homologous parental chromosomes. In rare cases, the common spirem (Pl. XII. Fig. 80) and diakinesis (Pl. XII. Fig. 82) are also observed.

Spindles appear very irregularly even as early as the stage of very young spirem (Pl. XII. Fig. 84, 85, 88). A large apparently homologous chromatic mass is sometimes seen enveloped in a spindle (Pl. XII. Fig. 86). A similar case has already been described as by constriction by Jeul ('00) and Tischler ('08, p. 93) in Syringa-hybrid, while by Farmer and Digby ('10, p. 205) in Polypodium-hybrid as abnormal mitosis, to which view I assent. At the time of synapsis the nucleus in a few cases appear as if to divide by constriction (Pl. XII. Fig. 76).

Normal equatorial plates can not be recognized, but many abnormal chromosomes are scattered irregularly in the spindle (Pl. XIII. Fig. 91, 92). The chromosome number is not definite (17–23), being almost always more than the expected number, 16. Juel ('00) and Tischler ('08, p. 87 ff.) observed the increase of chromosomes in the pollen mother cells of Syringa-hybrid. Although Gates ('07) held that chromosomes increased in number in Oenothera-hybrids (*O. lata* × *O. Lamarckiana*), he ('09) decided afterwards, that there was no increase in the chromosome number in this hybrid as he found later that it was in reality a hybrid between *O. lata* and *O. gigas*. Farmer and Digby ('10, p. 193) studying a Polypodium-hybrid, *P. aureum* (34 chromosomes) × *P. vulgare* var. *elegantissimum* (more than 95 chromosomes), observed 95–125 chromosomes (mostly 95–

(1) It is highly probable that the female organs are partially sterile. Studies on the next generation thus obtained are going on.

105). Also in our wheat × rye the chromosomes increased in number. The increase in number in our case takes place in the following manner. The spindle appears earlier than in normal cases, *i. e.*, before diakinesis. Large spirems drawn to the poles break into many pieces, thus the increase of chromosomes in number is caused, and at the same time their size and form are not uniform. By observing the bivalent chromosomes going to the pole and also the very long chromosomes in the spindle, it is made clear that the spindle formation begins at the time of spirem as shown in Fig. 81 of Pl. XII; and the mitosis is thereby abnormally accelerated. In these abnormal mitotic figures, I observed very often the segmented chromosomes, the curved spindles and many paired chromosomes (Pl. XIII. Fig. 91, 92). In some cases, the paired chromosomes are separated again by means of secondary spindle fibres (Pl. XII. Fig. 89). Besides the chromosomes, many chromatic granules are visible in the cytoplasm as observed by many cytologists in the plant hybrids (Pl. XII. Fig. 89; Pl. XIII. Fig. 98, 99, 100).

Judging from the deficiency of cytoplasm in the pollen mother-cell of the hybrid, it may be proved that when the quantity of chromatin reaches a definite ratio to that of cytoplasm, it urges the formation of the spindle. In many hybrids—Syringa, Bryonia, Polypodium, etc.—the augmentation of chromosomes and their scattered arrangement must have been produced by the abnormally premature formation of the spindle. The primary cause of the sterility of pollen seems to be due to the deficiency of cytoplasm, and this leads to the abnormal mitosis. The chromosomes which form the daughter nuclei, transform into spirem as in rye (Pl. XIII. Fig. 99). Besides the two daughter or four granddaughter nuclei, there is formed often a dwarf nucleus in the place where one or more chromosomes are left behind (Pl. XIII. Fig. 94).

In the second division only a large irregular chromatic mass is visible in the equatorial plate (Pl. XIII. Fig. 98) and a few smaller chromatic masses are found in other parts of the spindle.

There are besides some abnormal figures, such as three spindles in a pollen mother-cell (Pl. XIII. Fig. 93), or the case in which one daughter nucleus is in the resting state while the other is in mitosis (Pl. XII. Fig. 87).

In spite of the many irregular processes, the perfect tetrad can be observed in some loculi, chromosomes changing into spirems or fine granules, which are produced by the breaking up of the spirem. Six pollen grains are sometimes formed through three separate spindles in the common membrane of the mother-cell (Pl. XIII. Fig. 97). The pollens of the hybrid differ in size among themselves. The results of my present study confirm the statement,—that the large pollens have many chromosomes, while the smaller ones have a few,—which has been made by other authors in other cases.

The degenerating phenomena of the pollen cells are observed at every stage of their

developement, *e.g.*, in synapsis, spirem, the first division (Pl. XIII. Fig. 96), the second division (Pl. XIII. Fig. 95), etc. In the anaphase of the first division in the degenerating state, there are deeply stained granules, whose number is about equal to the expected number of the chromosomes (Pl. XIII. Fig. 96).

Summery.

1. The somatic nucleus, in barley, wheat, rye, and wheat-rye hybrid, has neither prochromosomes nor any other particular structures in the perfect resting state; but the prochromosomes gradually appear until they reach a definite number. Then other chromatin granules of smaller size come into view. Reticulum is then formed, completing itself gradually by connecting one chromatin granule with another by means of linin.

2. In the pollen mother-cells, the reticulum is also formed in the same way.

3. The reticulum, changing into nuclear threads as in barley and rye, or changed into threads as in wheat, enters into synapsis. In wheat, the nuclear threads arrange in pairs, in which the homologous chromomeres conjugate. In the synaptic ball the chromatic masses are often present.

4. In barley and rye, in synapsis, the reticulum turns into uniform nuclear threads containing chromomeres and arranged in pairs. These nuclear threads together with chromomeres conjugate at post-synapsis.

5. The conjugated spirem betrays its double nature afterwards by its separation into two twisted filaments.

6. The spirem may perhaps be continuous and may segment to form chromosomes.

7. The double chromosomes originated from paired spirems, are formed by separation and not by looping.

8. The first division is the reduction division and the second the equation division.

9. The number of chromosomes is 8 in wheat and rye, and 7 in barley.

10. In interkinesis or in tetrad nuclei the chromosomes alveolate in barley and wheat, while they transform into spirem in rye.

11. In the wheat-rye hybrid the formation of the reticulum in the pollen mother-cells and the contraction of the same into the synapsis take place in the same way as the original plants.

12. Synapsis lasts very long even to mitosis sometimes. It is perhaps caused by the difficulty of reciprocal action of parental chromosomes or by the insufficient nourishment furnished by cytoplasm or by both of these conditions.

13. Mitosis begins to take place before diakinesis, sometimes as early as the stage of young spirem. The spirem is segmented into chromosome-pieces, whose number is usually

larger than the expected number of chromosomes.

14. This abnormal premature formation of the spindle may be due to the deficiency in cytoplasm.

15. Many small chromatic granules are visible scattered in cytoplasm in mitosis.

16. The chromosomes which are distributed in the daughter or grand-daughter nucleus, transform into spirems as in rye.

17. The equatorial plates of the second division are generally composed of only one large chromatic mass attached to the spindle fibres. There are many other abnormal features in mitosis.

18. Degenerating phenomena are seen in the pollen and also in various stages of developement of the pollen mother-cell,—in the synapsis, in the spirem and in the first and second divisions.

I wish here to express my heartiest thanks to Prof. Dr. K. Shibata under whose direction these studies have been made, to Prof. Dr. K. Miyabe whom I am indebted for his kind help in completing this paper in the absence of Prof. Shibata, and to Prof. Dr. T. Minami who has encouraged me constantly. I wish also to acknowledge my indebtedness to Mr. Masanao Ito who has kindly allowed me to use freely the materials of the hybrid he had made between wheat and rye.

College of Agriculture, Tohotu Imperial University, Sapporo. Oct. 1910.

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Explanation of Figures in Plates X–XIII.

All the figures were drawn with the aid of an Abbe drawing apparatus and with a Zeiss achromatic homogeneous immersion 1/12 or with a Zeiss apochromatic 2 mm. objective.

The figures 28 and 31, in Pl. I; 50 and 52, in Pl. XI; 66, 67, 75, 76, 83, 84 and 89 in Pl. XII; and 94, 95, and 100 in Pl. XIII. were drawn with a Zeiss compensating ocular 12; the others with the ocular 18.

Pl. X. Fig. 1–31. Barley.

Fig. 1. Very young archesporium nucleus having no prochromosomes, being in the perfect resting state.

Fig. 2. The same having visible prochromosomes in a further stage.

Fig. 3. The same, chromosomes in formation.

Fig. 4. Nucleus in the somatic cell of a glume. Stage of prochromosomes, accompanied by small chromatin granules in a portion of nuclear cavity.

Fig. 5. The same, in chromosome-formation showing polarity.

Fig. 6. A tapetum nucleus showing 14 chromosomes.

Fig. 7–31. Nuclei of the pollen mother-cells.

Fig. 7—12. Prochromosomes becoming visible; and reticulum in formation.

Fig. 13. Synaptic contraction.

Fig. 14. Tangential-section of the same showing two parallel threads plainly.

Fig. 15. Two twisted threads before conjugation.

Fig. 16. Synaptic ball loosening up to hollow spirem.

Fig. 17. Tangential-section of a nucleus at the time of hollow spirem.

Fig. 18. Two twisted spirems in the so-called second contraction.

Fig. 19. Spirems segmented.

Fig. 20, 21. Chromosome-formation.

Fig. 22. Diakinesis.

Fig. 23, 24. Equatorial plate of the first division.

Fig. 25. Polar view of the equatorial plate.

Fig. 26. Anaphase.

Fig. 27. Chromosomes at the pole.

Fig. 28. Prophase of the second division.

Fig. 29. Polar view of the equatorial plate of the second division.

Fig. 30. Equatorial plate of the second division.

Fig. 31. Chromosomes alveolating to form tetrad nuclei.

Pl .X. Fig. 32—40, Pl. XI. Fig. 41—45.

Rye.

Fig. 32—35. Prochromosomes and reticulum formation.

Fig. 36. Synapsis contracting.

Fig. 37. "Chromatic bodies" migrating to the neibouring cells.

Fig. 38. Synapsis contracted.

Fig. 39. Tangential-section at the time of hollow spirem.

Fig. 40. Two twisted spirems separated after conjugation.

Pl. II. Fig. 41. Many small nucleoli in the hollow spirem stage.

Fig. 42, 43. Large spirem.

Fig. 44. Chromosome formation.

Fig. 45–47. Diakinesis.

Fig. 48. Multipolar spindle.

Fig. 49. Bipolar spindle

Fig. 50. Polar view of the first metaphase.

Fig. 51. Chromosomes changed into spirems in daughter nuclei.

Fig. 52. Polar view of the equatorial plate of the second division.

Fig. 53. Second metaphase.

Fig. 54. Chromosomes transforming into spirems in tetrad nuclei.

Pl. XI. Fig. 55–64. Pl. XII. Fig. 65 -70.

Wheat.

Fig. 55 - 57. Prochromosomes and reticulum in formation.

Fig. 58. Synapsis contracting in tangential-section.

Fig. 59. The same stage showing two parallel fine threads containing homologous chromomeres.

Fig. 60. Synaptic ball.

Fig. 61, 62. Hollow spirem.

Fig. 63, 64. Chromosome formation.

Pl. III. Fig. 65. Diakinesis.

Fig. 66. Metaphase.

Fig. 67. Alveolating chromosomes in a daughter nucleus.

Fig. 68. Metaphase of the second division.

Fig. 69. Polar view of the second equatorial plate.

Fig. 70. Alveolating chromosomes in tetrad nuclei.

Pl. XII. Fig. 71–90. Pl. XIII. Fig. 91–100.

Wheat-rye hybrids.

Fig. 71–74. From prochromosome stage to synapsis stage.

Fig. 75. Normal synaptic ball.

Fig. 76. Amitotic figure in synaptic stage.

Fig. 77. Tangential-section through later synaptic contraction showing its spirem structure.

Fig. 78–80. Two twisted spirems.

Fig. 81. Later large spirems.

Fig. 82. Diakinesis.

Fig. 83–86 and 88. Abnormal mitotic features, spindle appearing earlier.

Fig. 87. A daughter nucleus in rest and the other in mitosis.

Fig. 89. Chromosomes approaching the poles, and entering into a further stage of the secondary spindle.

Fig. 90. Long large spirems distributed in the spindle.

Pl. IV. Fig. 91, 92. Anaphase, chromosomes often pairing.

Fig. 93. Three spindles in a pollen mother-cell.

Fig. 94. Terophase, two double chromosomes are visible, and also a dwarf nucleus formed of the chromosomes left behind.

Fig. 95. Second mitosis in degeneration.

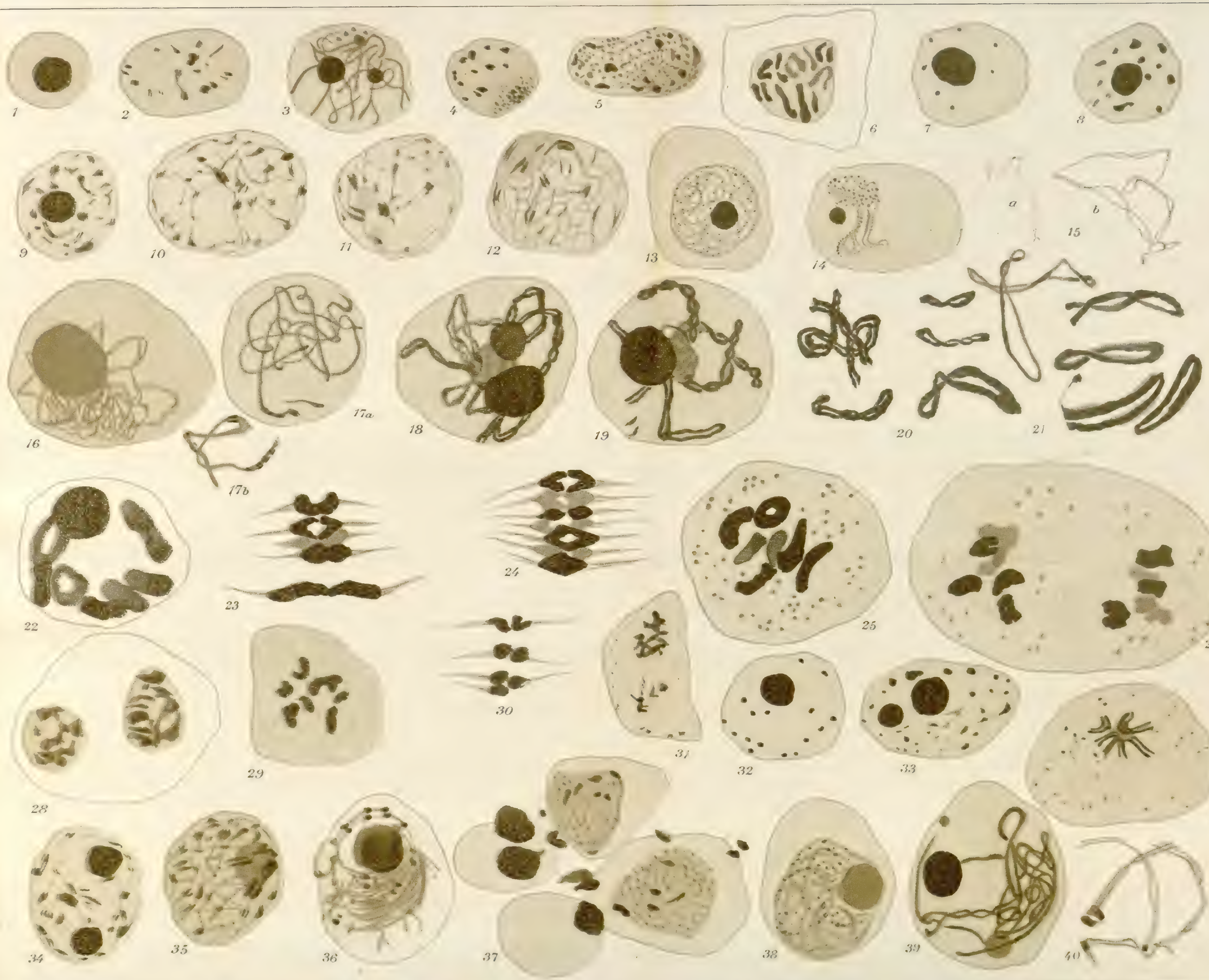
Fig. 96. First mitosis in degeneration.

Fig. 97. Six pollens formed in a mother cell in degeneration.

Fig. 98. Second division.

Fig. 99. Two daughter nuclei in resting state.

Fig. 100. Terophase of the first division. Many small chromatic granules scattered in cytoplasm.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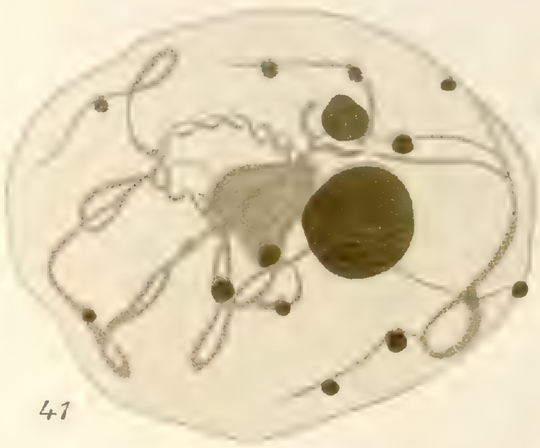
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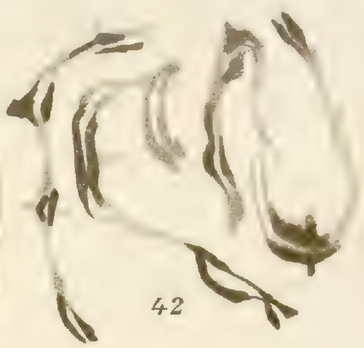
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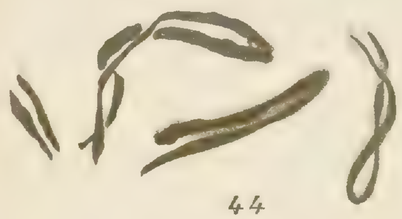
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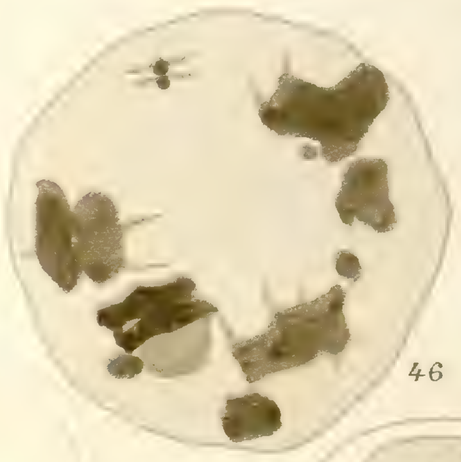
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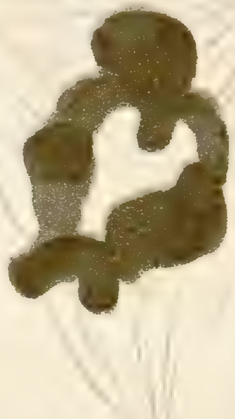
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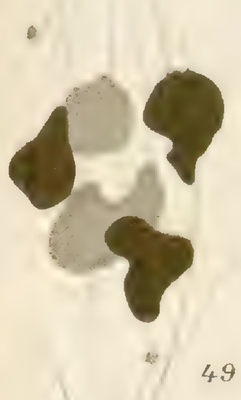
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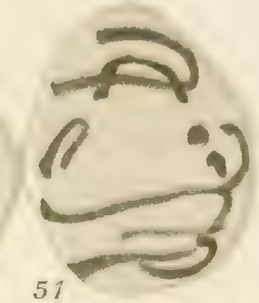
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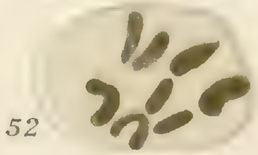
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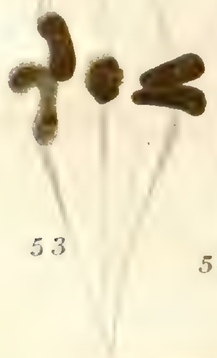
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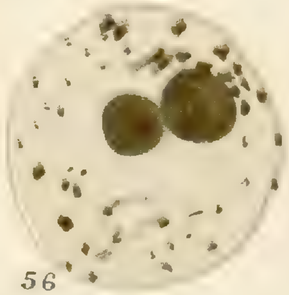
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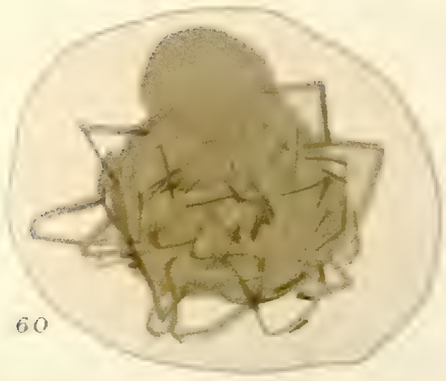
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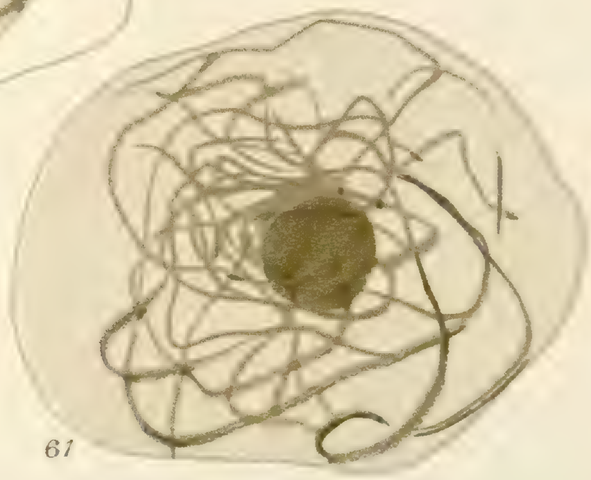
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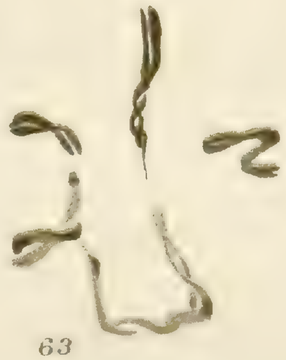
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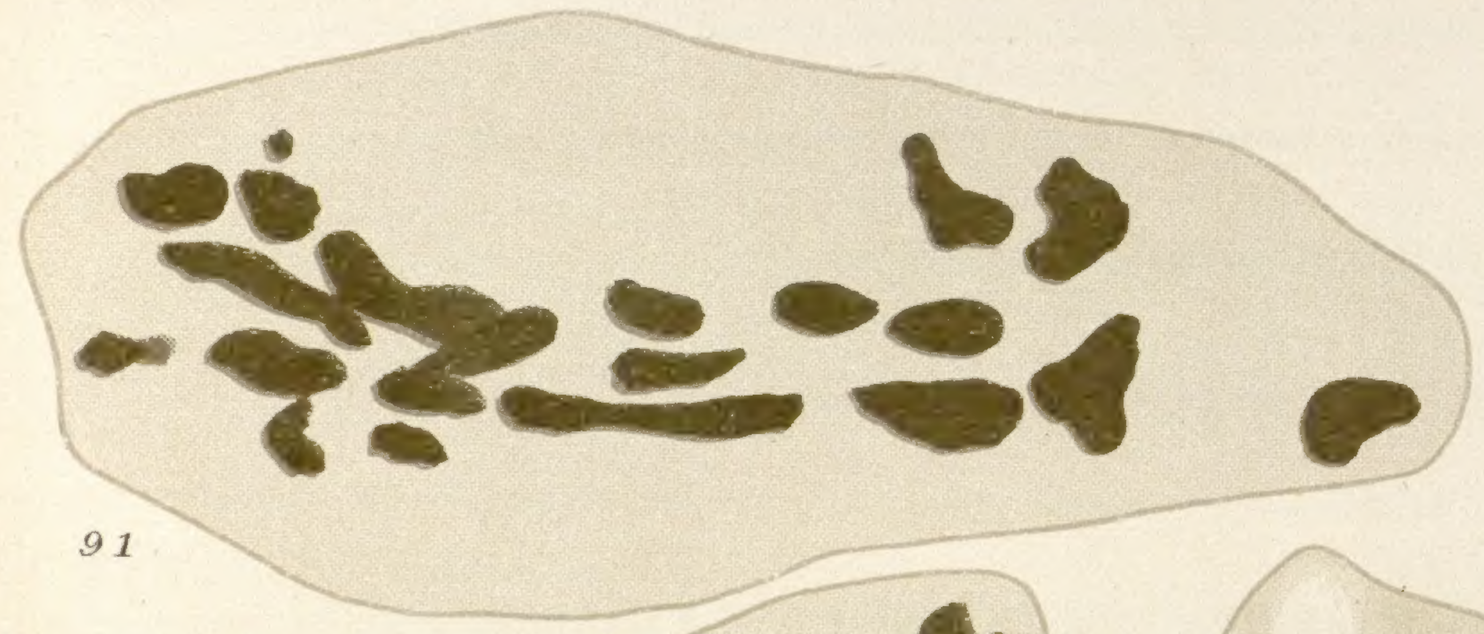

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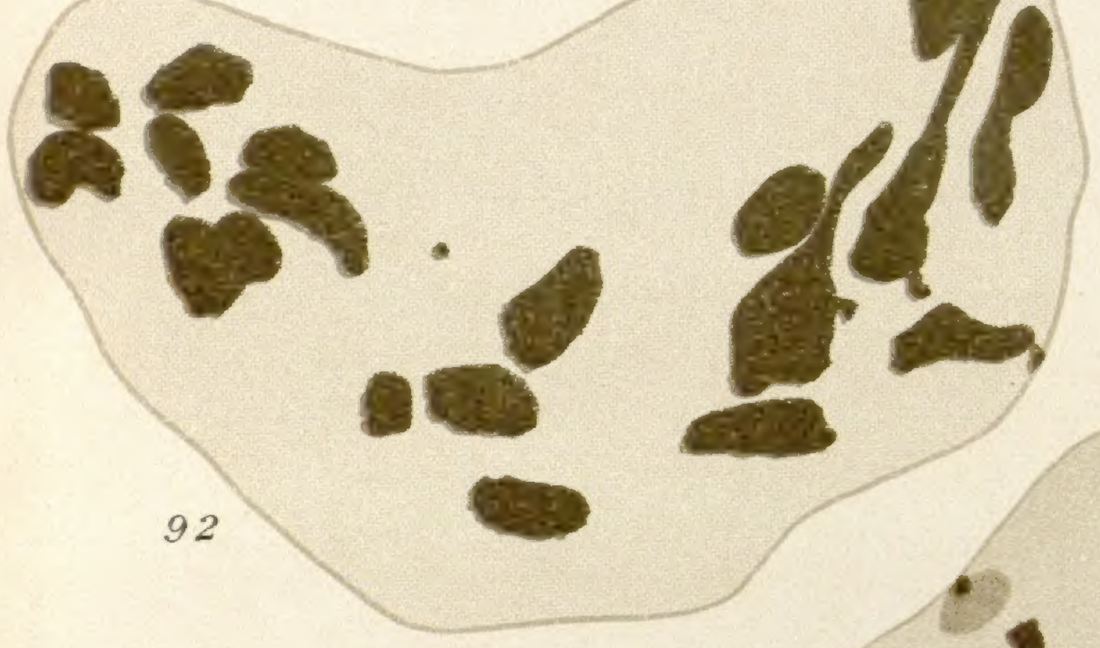

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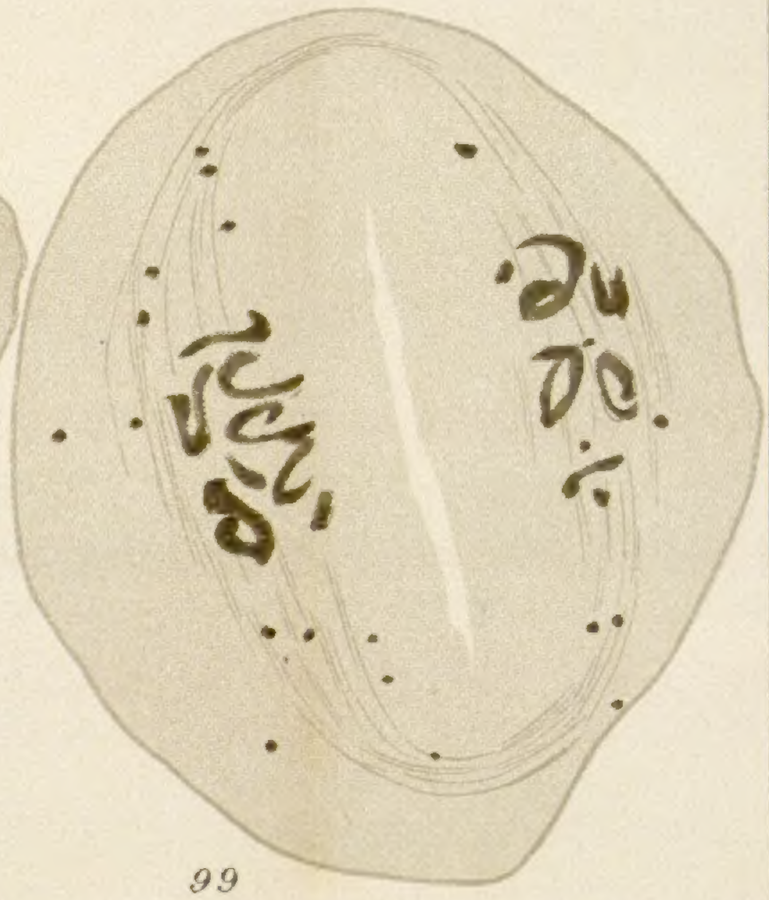

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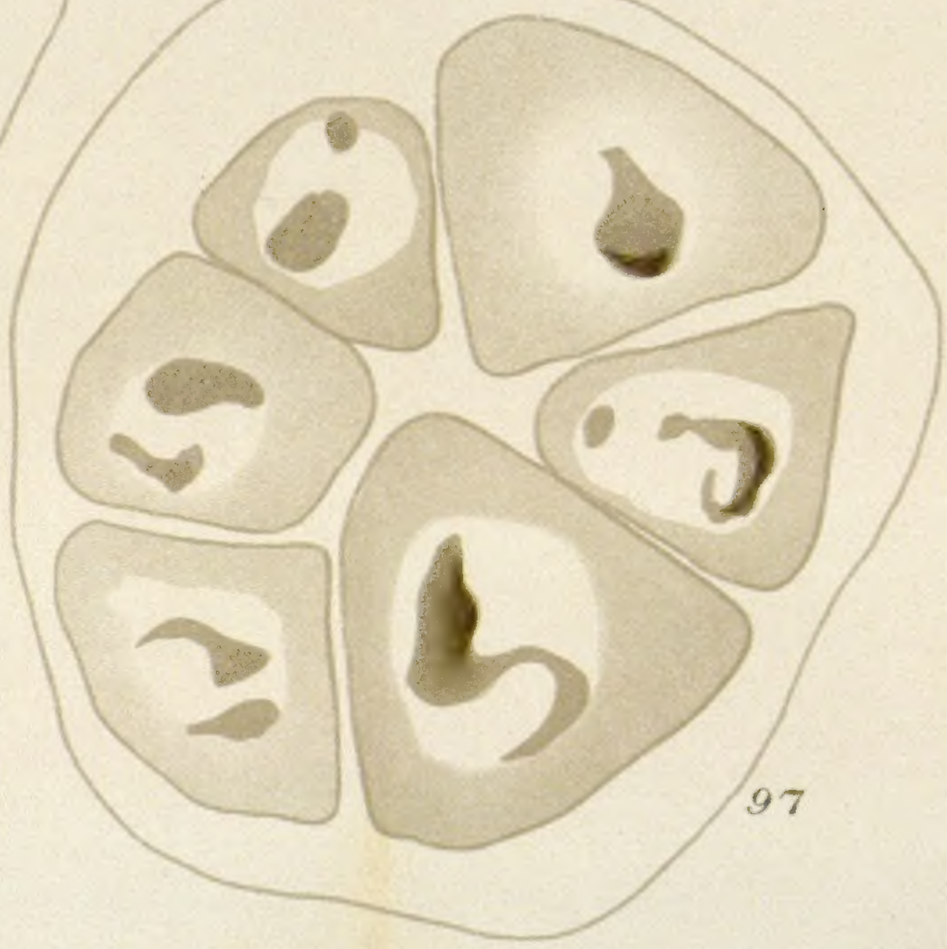

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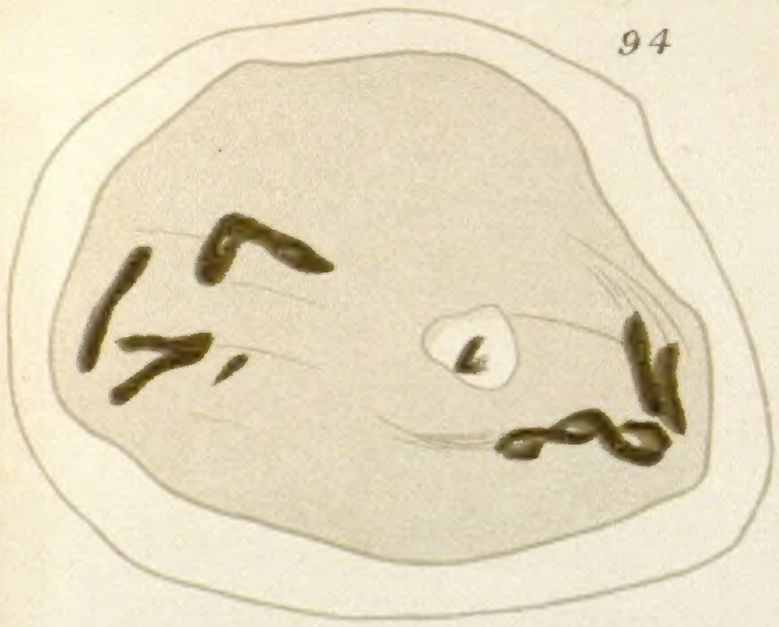

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